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Developmental timing of DNA elimination following allopolyploidization in wheat

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Running title

Tissue-specific DNA deletion

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Abstract

The elimination of DNA sequences following allopolyploidization is a well known phenomenon. Yet, nothing is known about the biological significance, the mechanism, or the precise developmental timing of this event. In this study, we have observed reproducible elimination of an *Ae. tauschii* allele in the genome of the second generation (S2) of a newly synthesized allohexaploid derived from a cross between *T. turgidum* and *Ae. tauschii*. We show that elimination of the *Ae. tauschii* allele did not occur in germ cells but instead, occurred during S2 embryo development. This work shows that deletion of DNA sequences following allopolyploidization might occur also in a tissue-specific manner.

Whole genome doubling has been remarkably prevalent during plant evolution (ADAMS and WENDEL 2005a; ADAMS and WENDEL 2005b; CHEN 2007; CHEN and NI 2006; FELDMAN *et al.* 1997; OSBORN *et al.* 2003; SOLTIS and SOLTIS 2000; WENDEL 2000). Indeed, it is now a truism that most flowering plants have experienced at least one polyploidy event at some point during their genomic history (WOLFE 2001), either through the doubling of a single genome (autopolyploidy) or, more commonly, by combining two or more distinct but related genomes (allopolyploidy). Not solely a process that characterizes ancient lineages, genome doubling rather remains a powerful mechanism for speciation even today.

Bread wheat, *Triticum aestivum,* is a hexaploid (2n= 6x= 42; genome BBAADD) that originated from hybridization events in *Triticum* and *Aegilops* involving three diploid progenitors that diverged from a common ancestor ~4 MYA (FELDMAN 2001; FELDMAN and LEVY 2005). The wheat group comprising *Aegilops* and *Triticum* thus offers an ideal system for the study of the evolution of polyploids given that most diploid progenitors are known, and because allopolyploids can readily be synthesized. In addition, hexaploid bread wheat is only ~10.000 years old (DUBCOVSKY and DVORAK 2007; FELDMAN and LEVY 2005), thereby permitting close genetic comparisons with resynthesized wheat and other polyploids.

Newly synthesized allopolyploids (amphiploids) can induce a variety of rapid and reproducible genomic changes, including the elimination of DNA sequences (ADAMS and WENDEL 2005a; GAETA *et al.* 2007; LIU *et al.* 1998a; LUKENS *et al.* 2006; MA *et al.* 2004; OZKAN *et al.* 2001; PIRES *et al.* 2004; SHAKED *et al.* 2001; SKALICKA *et al.* 2005). Yet, nothing is known about the biological significance, the mechanism, or the precise developmental timing of this phenomenon.

Both genome-specific (GSS) and chromosome-specific (CSS) DNA sequences have been shown, by gel blot analysis, to undergo elimination in newly formed wheat allopolyploids (FELDMAN *et al.* 1997; LIU *et al.* 1998a; LIU *et al.* 1998b; OZKAN *et al.* 2001). However, while gel blot analysis can show

that DNA elimination had indeed occurred, a study of the precise developmental timing of DNA elimination by this approach requires DNA material from germ cells and from embryonic tissues. Unfortunately, the limited amount of DNA available for isolation from these tissues precluded this possibility. Therefore, in the following study we instead used PCR analysis to identify polymorphic alleles of GSS and/or CSS in *Triticum* and *Aegilops* species.

Using this approach, we were able to successfully identify polymorphic PCR bands in DNA isolated from young leaves (age 4 weeks post-germination) of *T. turgidum* (accession TTR19) and *Ae. tauschii* (accession TQ27). A PCR band of 148 bp was produced in TTR19, while a shorter band of 95 bp was produced in TQ27 (Figure 1). The 148-bp TTR19 band and the 95-bp TQ27 band were extracted from the agarose gel, cloned, and sequenced. A sequence-based comparison of the two bands revealed that the DNA sequences of the two fragments were almost identical, except that 53 bp had been deleted from the shorter TQ27 band (see Figure 1 schematic diagram). This DNA sequence was identified in previous studies as a low copy number chromosome-specific sequence, mapped to the long arm of chromosome 3B (3BL) of common wheat (LIU *et al.* 2003; WEISSMANN *et al.* 2005). Here, we report on another homoeoallele of this locus from the DD genome (*Ae. tauschii,* accession no. TQ27). No sequences of significant similarity were found in the EST or cDNA databases, nor were any open reading frames (ORFs) detected in the common sequence, indicating that this DNA locus may contain a non-coding sequence.

The availability of a newly synthesized allohexaploid (S1 to S4 generations) derived from a cross between TTR19 and TQ27 (OZKAN *et al.* 2001) and the identification of polymorphic alleles in these two species facilitated analysis of the inheritance of both alleles following allopolyploidization. Both the TTR19 and TQ27 alleles were PCR amplified from the parental lines (TTR19 and TQ27) and from the first four generations (S1 to S4) of the newly synthesized derived allohexaploid (Figure 2). As such, we expected that both alleles would be inherited (i.e, present additivity) in the S generations, given that both

parents (TTR19 and TQ27) are derived from inbred lines (see OZKAN *et al.* 2001). Any deviation from additivity (i.e., a loss of heterozygosity) in any of the S generations would, therefore, most likely be due to DNA elimination. While the S1 is heterozygous, containing both the TTR19 and TQ27 alleles, the TQ27 allele was lost in the S2, S3, and S4 generations (Figure 2). Indeed, the same process was reproducible in independently made S1-S4 plants (see Figure 2 replicates). To insure that the absence of the TQ27 band in the S2, S3 and S4 generations was due to deletion and not the result of other mutations, the two bands obtained in S1 were isolated from the gel, cloned and sequenced. The sequences of the two bands were identical to the TTR19 and the TQ27 alleles isolated from the parental lines (Figure 1). Moreover, the loss of the TQ27 allele in the later generations was not the result of target-competition in the PCR amplification as both alleles could be detected when DNA from both parents was mixed and used as template in the PCR analysis (see Figure 1). The elimination of the TQ27 allele in S2 was also validated by Southern Blot analysis (supplemental Figure 1). Note that chromosome number was determined in newly synthesized allohexaploids and only those having the expected euploid chromosome number were analyzed. Supplemental Figure 2 shows the karyotype of S3 individuals indicating that no chromosome loss was occurred during the development of S2 plants. Thus the elimination of the TQ27 allele in leaves of S2 plants was not due to chromosome loss.

Our observation of loss of the TQ27 allele using PCR analysis facilitated the study of the exact developmental timing of the allelic elimination. One of two possible scenarios can explain the timing of DNA elimination: **(1)** DNA deletion can occur in germ cells, or **(2)** DNA deletion can occur in somatic cells during development of the embryo or development of the plant. Both possibilities were tested by isolating DNA from: **(1)** pollen grains, ovaries, and the first true leaf of S1 plants, and **(2)** immature seeds (two weeks after fertilization; not shown), mature embryos (Figure 3B-1), first roots (Figure 3B-2), and first leaves (Figure 3B-3) of S2 plants. Samples from later generation plants were not considered since TQ27 allele elimination was observed in DNA isolated from the young leaves (1 month after germination) of S2 plants,

yet with the allele being present in S1 plants. The results show that the TQ27 allele was present in tissues from pollen, ovaries, and the first true leaf of S1 plants as well as from the immature seeds and mature embryos of S2 plants (Figure 3A). In addition, the TQ27 allele was detected in the first root of S2 seedlings (Figure 3A). Because the TQ27 band corresponded to only a weak signal in S2 tissues (marked by arrows in Figure 3A -upper panel), some PCR products were visualized in 4% acrylamide gel for better resolution (Figure 3A -lower panel). TQ27 allele elimination was initially observed in the first leaf of S2 seedlings (Figure 3A), indicating that the deletion of the allele to be tissue-specific. However, important to note that our analysis was based on 'all or nothing' elimination of the TQ27 allele. Thus, it is clear that the TQ27 band was totally lost in the first leaf of the S2 seedlings (Figure 3A). In any case, to insure that the TQ27 allele has eliminated in leaves but not in roots, a quantitative PCR (qPCR) analysis was performed on the parental lines and 4 weeks old roots and leaves of S2 generation. The qPCR clearly showed that the elimination indeed occurred in a tissue-specific manner (supplemental Figure 3).

In summary, although we can not draw a general conclusion, this study shows that elimination of DNA sequences following allopolyploidization may also occur in a tissue-specific manner. To insure that the tissue-specific deletion affect other DNA sequences, we performed AFLP analysis on the parental lines and 4 weeks old roots and leaves of S2 generation and found that DNA fragments either underwent deletion in leaves only indicating tissue-specificity (Figure 4, cases1-3), or deleted from both roots and leaves (Figure 4, cases 4-6). The mechanism of DNA loss following genome doubling nonetheless remains unknown because the deletion breakpoints have yet to be identified. However, it is important to mention that the deletion of the 53 bp that gave rise to the allelic variation between TTR19 and TQ27 occurred within short inverted repeats (ATGCTT) at both ends of the deleted sequence (Figure 1 schematic diagram). Future studies should thus focus on the identification of the deletion breakpoints to elucidate the mechanism(s) of DNA elimination following allopolyploidization and to reveal whether this phenomenon has any significance from an adaptive perspective.

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Figures and Figure legends

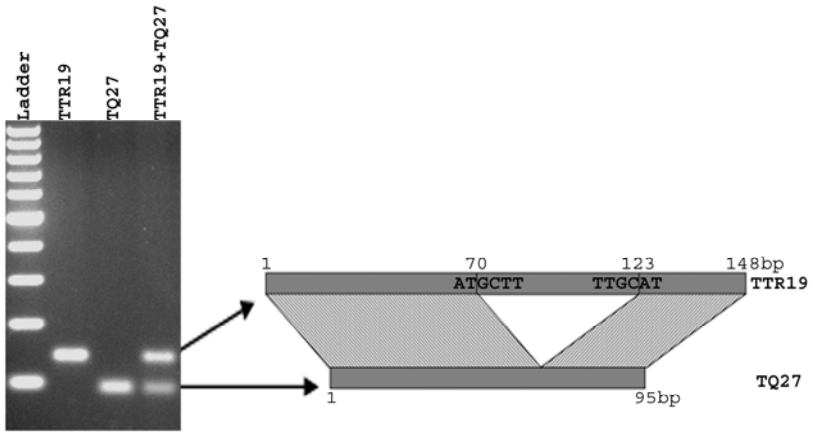

Figure 1. Identification and characterization of homoeoalleles in *T. turgidum* (TTR19) and *Ae. tauschii* (TQ27) by PCR analysis, using the following primers: 5'-AGCAACCATAAATTGGACTTTCA-3'; 5'-CATTGTTCTGTGCTTTTTCTCG-3'. The PCR conditions were as follows: 3 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 35 s at 58 °C, 1 min at 72 °C, and then 7 min at 72 °C. The PCR products were visualized in 2% agarose gel. The 'TTR19+TQ27' lane is mix of DNA from the two species used as PCR template so as to eliminate the possibility of target-completion in the reaction. A 100-bp DNA ladder (Fermentas) was used. The scheme on the right represents a molecular characterization of the two alleles showing 148 bp from TTR19 and 95 bp from TQ27. The shaded areas represent zones of identical DNA sequence between the two alleles; the 53-bp deletion from the TQ27 allele lays between positions 70 and 123 of the TTR19 allele. The DNA sequences of the deletion breakpoints are shown as well.

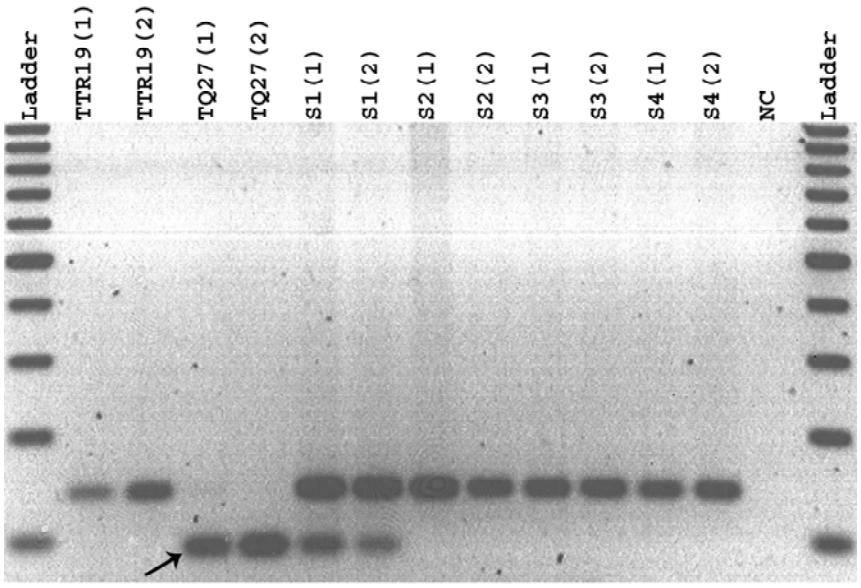

Figure 2. Elimination of the TQ27 allele (indicated by an arrow) in the S2, S3, and S4 generations, as depicted by PCR analysis, using the same primers as in Figure 1. S1(1), S2(1), S3(1), and S4(1) were derived from a cross between TTR19(1) and TQ27(1), while S1(2), S2(2), S3(2) and S4(2) were derived from a cross between TTR19(2) and TQ27(2). Note that 5-8 individuals for each generation were tested and the results were repeatable in all of the individuals. In a negative control (NC) reaction, H_2O was used as template in PCR, performed using the same primers. A 100-bp DNA ladder (Fermentas) was used.

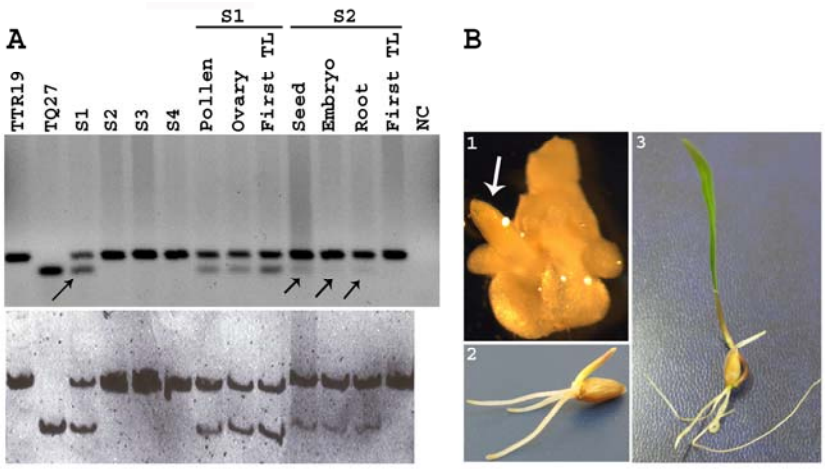

Figure 3. Developmental timing of TQ27 allele elimination. **A.** The TQ27 allele (indicated by an arrow) is present in S1 but absent from S2, S3, and S4 plants (first 6 lines from the left), as shown in the results of PCR analysis performed using the same primers as in Figure 1. The TQ27 allele is present in S1 tissues (pollen, ovary and first true leaf (First TL) and in certain S2 tissues, including seed, embryo, and first roots (indicated by arrows), yet it is absent from the first true leaf. In a negative control (NC) reaction, H_2O was used as a PCR template, together with the same primers as used above. The TQ27 band is clearly seen in seed, embryo, and root samples of S2 plants and individual tissues. The same PCR products were visualized in 4% acrylamide gel (lower panel). **B.** S2 plant tissues, from which DNA was extracted and analyzed by PCR: (1) germinated embryo; arrow indicates the initiation of the shoot; (2) first roots and (3) first true leaf.

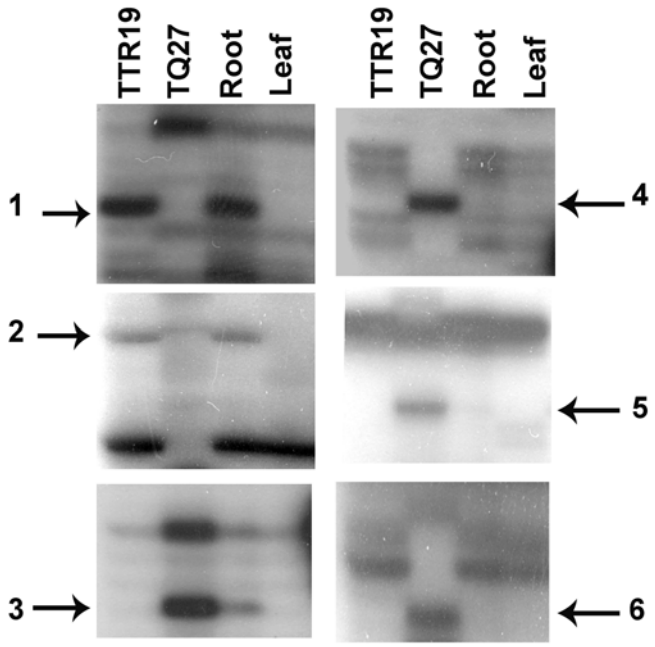

Figure 4. Tissue-specific DNA elimination as seen by AFLP analysis. Panels 1-2 indicate elimination of AFLP fragments in leaves but not in roots, while panels 4-6 indicate elimination in both roots and leaves. AFLP was performed as previously reported (Shaked et al., 2001).